research plan

[Abbreviated version – 8/31/07]

Dosimetric and Toxicologic Assessment of Amphibole Fiber-Containing Material from Libby, Montana

Peer Reviewed Revised Draft

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In Consultation with
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> The research for LA (Libby Amphibole) will be framed from the perspective that data are needed to characterize key features which determine inhaled internal dose and the resultant tissue reactions leading to pathogenesis.

> Other key areas of research will also inform the risk assessment, including inherent toxicity of LA relative to other forms of asbestos and differential susceptibility, including that of different life stages.

> Simulation exercises using modeled estimates of internal dose can be applied to the existing human data to help improve and refine our understanding of exposure-response relationships, which are based solely on external air measurements at this time

> Thus the proposed toxicology studies will help define key determinants of internal dose such as *in vivo* clearance rates.

> The studies will also be performed in a comparative fashion with respect to different types of asbestos, including negative controls.

Overall Research Approach

• (NHEERL) will conduct a series of *in vitro* dissolution and toxicity studies of LA and other fiber types to examine a variety of endpoints. When integrated with the subchronic inhalation exposure and other *in vivo* studies, such *in vitro* studies offer the potential to compare and examine a variety of other materials more quickly and inexpensively.

• NHEERL will conduct a series of short term comparative toxicity studies through intratracheal instillations of rats and mice, focused on intermittent exposures that are likely to be more representative of actual exposures experienced by residents.

• NHEERL will conduct a subchronic inhalation exposure study in the rat, examining a variety of toxicological endpoints up to 2 years after exposure, and the relationship between duration of exposure and the nature and persistence of effects.

• NHEERL will construct a dosimetry model to refine dose response and dose effect estimates for humans and to facilitate comparisons between humans, rats, and mice. *In vitro* dissolution data will be a key element in those models.

1. ***In Vitro* Dissolution Assays**
2. **Use of *In Vitro* Toxicology to Compare the Potency of Different Test Materials**
3. **Comparative Toxicology in Rats**
4. **Inhalation Toxicology in Rats**
5. **Dosimetry Model Development and Simulation Studies**

RESEARCH PROJECTS

1. *In Vitro* Dissolution Assays

Goal

The purpose of these *in vitro* studies is to provide data on key physicochemical parameters of clearance mechanisms to refine the dosimetry model predictions of retained dose, which in turn will support the risk assessment. Establishing the dissolution rates and distribution of fiber sizes after incubation with biological fluids will also provide insight on potential pathogenesis and allow relative potency comparisons with similar studies of other types of fibers.

Background

The purpose of these studies is three-fold: (1) to obtain a rate parameter for LA dissolution, an important physical clearance mechanism used in the fiber clearance model; (2) to provide a dataset specifically for LA that can be used to assess its relative biopersistence compared to other types of fibers and to previous studies conducted both *in vitro* and *in vivo*; and (3) to provide data for quantitative inference and dosimetric modeling of the proposed intra-tracheal instillation and inhalation studies.

Incubations will be performed using both SLF (simulated lung fluid) and acid solutions.

2. Use of *In Vitro* Toxicology to Compare the Potency of Different Test Materials

Goal

The purpose of these assays is to compare the ability of asbestos obtained from several sources to cause significant biological effects in cultured cells. The *in vitro* approaches using human cells will focus on respiratory tract epithelial cells and because these are the cells that first come in contact with inhaled substances such as asbestos. These *in vitro* studies are a rapid, inexpensive way to compare the relative potency of many different types and sizes of and inform the design of animal instillation and inhalation studies. They will also be able to provide information about predictive and clinical biomarkers, and the mechanisms by which different asbestos fibers cause toxicity.

3. Comparative Toxicology in Rats

Goal

The purpose of this series of intratracheal instillation studies is to provide mechanistic understanding of the comparative toxicity of different types of fibers, fiber-translocation kinetics, and age and disease-related susceptibility *in vivo*. Data obtained from these studies will be used to support the risk assessment and provide information regarding comparative toxicity of different fiber types. This approach will allow one to test multiple materials at varying concentrations

simultaneously. Additionally these studies provide important data on parameters for refining clearance rates used in the dosimetry model,

Experimental Approach

Specific Aim 1: Determine the dose-response relationships of LA in rats.

Specific Aim 2: Compare the toxicity of LA relative to other test materials.

Specific Aim 3: Determine if LA translocates to extrapulmonary organs in rats.

Specific Aim 4: Evaluate whether neonatal intratracheal instillation exposures are associated with increased injury, impairment of lung growth, or increased sensitivity to develop tumors.

Specific Aim 5: Evaluate whether underlying genetic susceptibility to develop diseases predisposes rats and mice to exacerbated adverse effects of LA.

We propose to use healthy Wistar Kyoto (WKY) and spontaneously hypertensive (SH) rats and healthy and atherosclerosis-prone Apo E knockout mice for determination of the mechanisms of enhanced susceptibility to pulmonary and systemic inflammation, oxidative stress, and mesothelioma.

4. Inhalation Toxicology in Rats

Goal

This project provides data on the relative potency of inhaled Libby amphibole (LA) compared to UICC amosite, a known fibrogenic and carcinogenic amphibole asbestos fiber. In addition to providing information on the intrinsic toxicity of LA, these inhalation studies provide data on the inhalability of LA and its initial deposition distribution which is necessary for refined dosimetry parameters (e.g., transport and translocation rates) and accurate retained dose predictions. Data obtained from these studies will support the risk assessment and identification of potential biomarkers.

Background

To accomplish the direct comparison of toxicity of LA fibers to amosite, we will conduct a subchronic 90-day nose-only inhalation exposure of male Fisher 344 rats followed by no-exposure recovery periods up to one year post-exposure (up to 2 years if resources permit). This study will provide dosimetry and toxicity information on fibers used in physiologically relevant inhalation exposures, with intensive measures of fiber burdens, clearance, pathology, and recovery

5. Dosimetry Model Development and Simulation Studies

Goal

The purpose of this project is to develop a dosimetry model to predict fiber deposition and retained fiber burden in rodents (rats and mice) and humans.

Specific Aim 1: Create a model of fiber deposition in rats and humans.

Specific Aim 2: Implement the deposition model and upgrade the MPPD software.

"Simulations for specific scenarios as needed by EPA Region 8 for immediate risk assessment applications will be performed and reported."

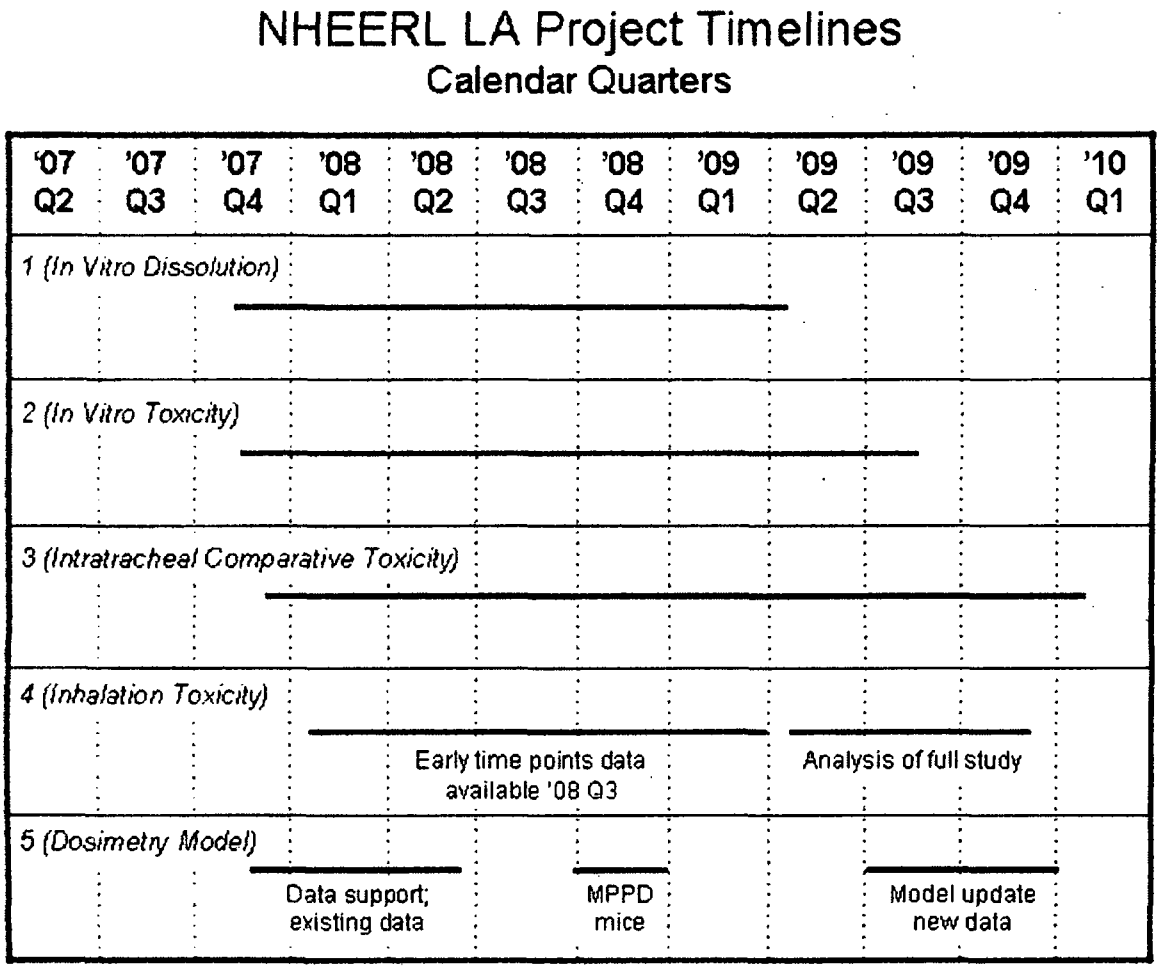
Specific Aim 3: Extend the model to describe fiber clearance in rats and humans.

Specific Aim 4: Implement the clearance model and upgrade the MPPD software.

Specific Aim 5: Develop a model of fiber deposition and clearance in mice.

Specific Aim 6: Implement the mouse model and upgrade the MPPD software.

Relative Timeline of Research Activities



Estimated Project Costs and FTE Support

	Costs	Principal Inv. FTE	Technical FTE
Overall Project Management		0.35	
Project 1 (*In Vitro* Dissolution)			
TEM sample preparation costs	$270,000	0.40	0.50
SLF equipment and supplies	$34,000		
SEEP ($70K x 2 yr)	$140,000		
Statistical Analysis and NHEERL Database Support	$60,000	0.30	
Subtotal	**$444,000**	**0.70**	**0.50**
Project 2 (*In Vitro* Toxicity)			
Postdoc ($75K x 3 yr)	$225,000	0.25	0.30
Student contractor ($30K x 2 yr)	$60,000	0.25	0.30
Cell culture supplies; genomic and proteomic analysis	$110,000		
TEM sample preparation costs	$40,000		
Subtotal	**$435,000**	**0.50**	**0.60**
Project 3 (Comparative Toxicology)			
Mice and rats	$70,000		
Postdoc ($75K x 3 yr)	$225,000	0.30	
Student ($40K x 2 yr)	$80,000	0.30	
SEEP ($70K x 1.5 yr)	$105,000		
Gene chips	$50,000		0.30
Lab supplies	$87,500		1.20
TEM sample preparation costs	$200,000		0.25
Pathology	$37,000		
NHEERL funds for 2 additional fibers	$210,000		
Subtotal	**$1,064,500**	**0.60**	**1.75**
Project 4 (Inhalation Toxicology in Rats)			
Contract subchronic nose-only inhalation exposure	$912,000	0.25	
TEM sample preparation costs	$200,000		0.25
Subtotal	**$1,112,000**	**0.25**	**0.25**
Project 5 (Dosimetry Model)			
Existing data - Contract modeler, programmer	$100,000	0.20	
Update with new data - Contract modeler, programmer	$30,000	0.15	
Mouse MPPD program - Contract modeler, programmer	$20,000	0.15	
Subtotal	**$150,000**	**0.50**	**0.00**
Total Project Costs	**$3,265,500**	**2.90**	**3.10**